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Effects of cyantraniliprole on biological characteristics and the related enzyme activities in *Plutella xylostella* (Lepidoptera: Plutellidae)

LIU Xia, WANG Hong-Yan[2], XIA Xiao-Ming[1], QIAO Kang[1], WANG Kai-Yun[1,*]

(1. College of Plant Protection, Shandong Agricultural University, Tai'an, Shandong 271018, China;
2. Cotton Research Center, Shandong Academy of Agricultural Sciences, Jinan 250100, China)

Abstract: 【Aim】 The diamondback moth (DBM), *Plutella xylostella* (L.), is one of the most important pests of cruciferous plants in the world. Cyantraniliprole is a novel anthranilic diamide insecticide registered for the control of lepidopteran pests, but there is sparse information available regarding its effects on *P. xylostella*. Here, we studied the effects of cyantraniliprole on biological characteristics and the related enzyme activities of *P. xylostella*. 【Methods】 The leaf-dipping method was used to test the toxicity of cyantraniliprole to the 3rd instar larvae of *P. xylostella*, and the LC_{20} and LC_{50} values were determined. The 3rd instar larvae were transferred onto cabbage leaves soaked in LC_{20} and LC_{50} of cyantraniliprole solution [20% lethal concentration (LC_{20}) = 0.03 mg/L and 50% lethal concentration (LC_{50}) = 0.08 mg/L] and allowed to feed for 48 h, then the biological characteristics such as the pupal duration, pupation rate, adult emergence rate and so on were recorded, and the related enzyme activities were also measured. 【Results】 The results showed that the estimated LC_{20} and LC_{50} values of cyantraniliprole on the 3rd instar larvae in leaf dip bioassay were 0.03 mg/L and 0.08 mg/L, respectively. Cyantraniliprole at LC_{20} and LC_{50} concentrations (LC_{20} = 0.03 mg/L, LC_{50} = 0.08 mg/L) could extend the duration of the 4th instar and pupal stage, and reduce the pupal weight and fecundity of *P. xylostella*. In treated groups, the pupation rate ($F = 42.19$; $df = 2, 6$; $P < 0.05$) and adult emergence rate ($F = 13.15$; $df = 2, 6$; $P = 0.006$) were significantly lower than those in the control group, respectively. Cyantraniliprole exposure also significantly increased the activities of protective enzymes (CAT and POD) at 12 h after treatment, but caused no effect on the activities of detoxification enzymes (CarE: $P = 0.43$; GST: $P = 0.54$; ODM: $P = 0.25$). In contrast, cyantraniliprole exposure increased the activities of detoxification enzymes at 48 h after treatment, but caused no effect on the activities of protective enzymes (CAT: $P = 0.58$; POD: $P = 0.13$). 【Conclusion】 The LC_{20} and LC_{50} concentrations of cyantraniliprole have negative effects on the growth and development of *P. xylostella*, which could reduce the population density of the next generation. Moreover, cyantraniliprole at these two concentrations could induce the activities of the related detoxifying enzymes of *P. xylostella*. This finding is of great significance in guiding the use of this insecticide in field and delaying the development of insecticide resistance.

Key words: *Plutella xylostella*; cyantraniliprole; toxicity; enzyme activity; biological characteristics

1 INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a major cosmopolitan pest of *Brassica* and other crucifer crops all over the world (Talekar and Shelton, 1993; Fathi *et al.*, 2011). The total annual cost for *P. xylostella* control throughout the world surpasses 4 billion US dollars (Zalucki *et al.*, 2012). But the extensive and inappropriate application of commercial insecticides has put *P. xylostella* under the high chemical selection pressure (Zhou *et al.*, 2010), and therefore led to high resistance to most commonly used insecticides such as β-cypermethrin, monosultap and phoxim (Zhou *et al.*, 2011), indoxacarb (Sayyed and Wright, 2006), avermectins (Pu *et al.*, 2010), and spinosad (Zhao *et al.*, 2002; Sayyed *et al.*, 2004).

The anthranilic diamide insecticides are a new class of insecticides to control *P. xylostella* with a novel mode of action targeting the ryanodine receptors in insect muscle cells (IRAC mode of action classification, group 28) (IRAC, 2007; Sattelle

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作者简介：刘霞，女，1988 年生，山东潍坊人，博士研究生，研究方向为农药毒理与有害生物抗药性，E-mail：liuxia.20072007@163.com

* 通讯作者 Corresponding author, E-mail：kywang@sdau.edu.cn

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et al., 2008). Chlorantraniliprole (rynaxypyr), the first commercially available anthranilic diamide, registered for use in the USA in 2008, exhibits anti-feedant activity against chewing insects (DuPont, 2007). Cyantraniliprole (cyazypyr), a new diamide insecticide, shows exceptional insecticidal activity on a broad range of Lepidoptera, Coleoptera, Diptera, and Isoptera insects, and it is reported to be active against a broader spectrum of insects than chlorantraniliprole (Chai *et al.*, 2010). So the wide application of cyantraniliprole throughout the world would be seen in the future and the market of cyantraniliprole will be also prosperous.

Cyantraniliprole has been registered to control *P. xylostella*, *Spodoptera litura*, *Pieris rapae*, *Spodoptera exigua*, *Phyllotreta striolata* and *Liriomyza sativae*. However, a systematic study of cyantraniliprole has not been reported to date. The objective of the present study was to investigate the effects of cyantraniliprole on *P. xylostella*, including the physiological effects and biochemical effects. Physiological effects included larval and pupal development, pupal weight, pupation and adult emergence rate, adult longevity, fecundity and egg hatch rate. Biochemical effects mainly referred to the changes of enzyme activities such as catalase, peroxidase, cytochrome P450-dependent monooxygenases, glutathione *S*-transferases and hydrolases in *P. xylostella*. Such information is important for a better use of cyantraniliprole in *P. xylostella* management.

2 MATERIALS AND METHODS

2.1 Test insect

The field *P. xylostella* strain was collected in May 2012 from Shandong Province of China, where pesticides had not been used for years. This strain has been maintained in the laboratory without exposure to any insecticide. The larvae were reared routinely upon improved vermiculite-cultured radish seedlings, while adults were provided with 10% honey solution in the laboratory. The strain was kept in an insectary under the conditions of 25 ± 1℃, 35% - 55% RH and a long photoperiod of 16L∶8D. All experiments unless otherwise stated were conducted under these conditions.

2.2 Test insecticides

Cyantraniliprole (10% WP) was obtained from Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), China. Chlorantraniliprole (200 g/L SC) was obtained from Shanghai Shengnong Pesticide Co. Ltd, China.

2.3 Leaf-dip bioassays

The bioassays were conducted by using a leaf-dipping method (Shelton *et al.*, 1993; Liang *et al.*, 2003). By using two-fold dilutions, seven concentrations of cyantraniliprole ranging from 0.64 to 0.01 mg/L were prepared with distilled water while chloran-traniliprole was diluted from 2.56 to 0.04 mg/L. Fresh cabbage leaves were cut into the same size discs, which were dipped in different concentrations of cyantraniliprole or chlorantraniliprole for 10 s, dried at room temperature, and then individually transferred to plastic petri dishes (9.4 cm in diameter, and 2.0 cm in depth) where 20 3rd instar larvae were inoculated in each dish. Seven concentrations plus one control (leaf discs treated with water alone) and three replicates were set up. Mortality was recorded after 48 h exposure.

2.4 Effects of cyantraniliprole exposure on biological characteristics of *P. xylostella*

Two insecticide treatments with cyantraniliprole [20% lethal concentration (LC_{20}) = 0.03 mg/L and 50% lethal concentration (LC_{50}) = 0.08 mg/L] and one distilled water control were set up in this study. Each treatment included three replicates. Cabbage leaves were dipped in cyantraniliprole solution at LC_{20} and LC_{50} equivalent concentrations and the water control. Two hundred uniform 3rd instar larvae were starved for 2 h prior to exposure to treated leaf disks, and then allowed to feed for 48 h. The surviving larvae were fed with fresh untreated cabbage leaves when necessary and allowed to pupate. One-day old pupae were weighed individually. The duration of the 4th instar and pupal stage, the pupal weight, the pupation rate and the adult emergence rate were recorded. For the fecundity study, one male and one female emerging from the plastic petri dishes were introduced into a finger tube (2.5 cm in diameter, and 8 cm in depth) whose inner wall was encircled with one layer of weighing paper. The open end of the tube was plugged with absorbent cotton soaked in a 10% sugar solution for providing nutrition to the adults, and a piece of cabbage leaf was inserted to stimulate oviposition. The eggs laid on the weighing papers and leaves were counted daily. The weighing papers and leaves were replaced every day until the death of the females. The longevity of adult males and females and the egg hatching rate were recorded as well.

2.5 Effects of cyantraniliprole exposure on enzyme activities in *P. xylostella*

Concentrations of cyantraniliprole used for assaying enzyme activities in *P. xylostella* were the same with those for tests of biological characteristics.

Cabbage leaves dipped in cyantraniliprole solution at LC_{20} and LC_{50} equivalent concentrations and the water control were used to feed the 3rd instar larvae for 48 h. During this period, the enzyme activities in *P. xylostella* at 12 h, 24 h and 48 h were measured, respectively.

2.6 Enzyme assays

2.6.1 Catalase (CAT) assay: Catalase activity was assayed by the method of Aebi (1984). A total of 90 larvae were homogenized in 5 mL of ice-cold phosphate buffer (50 mmol/L, pH 7.0) and then centrifuged at 12 500 g for 20 min at 4℃. The resulting supernatant was collected as the enzyme source. The reaction mixture consisted of 50 mmol/L hydrogen peroxide (H_2O_2) in 50 mmol/L phosphate buffer (pH 7.0) in a final volume of 5 mL. The reaction was started by adding the enzyme extract. The OD value at 240 nm was recorded at a 30-s interval for 5 min. The catalase activity was valued by the decrease in absorbance of hydrogen peroxide per minute.

2.6.2 Peroxidase (POD) assay: The peroxidase activity was determined by a colorimetric method, based on the change of absorbance at 470 nm due to the formation of tetraguaicol, the product of the guaiacol oxidation (Doerge *et al*., 1997). A total of 90 larvae were homogenized in 5 mL of ice-cold phosphate buffer (100 mmol/L, pH 6.0) and then centrifuged at 12 500 g for 20 min at 4℃. The resulting supernatant was collected as the enzyme source. Peroxidase assay medium contained 1.8 mL of phosphate buffer (100 mmol/L, pH 6.0), 0.02 mL of enzyme preparation, 0.1 mL of the guaiacol solution (100 mmol/L), and 0.1 mL of H_2O_2 solution (2.0 mmol/L). The OD value at 470 nm was recorded in a 30-s interval for 5 min.

2.6.3 Carboxyl esterase (CarE) assay: CarE activity was measured using α-naphthyl acetate (α-NA) as substrate according to the method described by Van Asperen (1962). A total of 20 larvae were homogenized in 5 mL of ice-cold phosphate buffer (40 mmol/L, pH 7.0) and then centrifuged at 12 500 g at 4℃ for 20 min. The resulting supernatant was collected as the enzyme source. The reaction mixture, consisting of 5 mL of 0.3 mmol/L α-NA and 0.1 mmol/L physostigmine, 1 mL of enzyme source, was incubated in a water bath at 30℃ for 15 min and stopped with 1 mL staining solution (0.2 g fast blue-B salt in 20 mL distilled water plus 50 mL 5% sodium dodecyl sulphate). The absorbance of the complex at 600 nm was read against blanks after 30 min. The CarE activity was determined from the α-naphthol production according to an experimentally determined standard curve.

2.6.4 Glutathione S-transferase (GST) assay: Glutathione S-transferase activity was estimated according to the method of Oppenoorth *et al*. (1979). A total of 30 larvae were homogenized in 5 mL of ice-cold phosphate buffer (66 mmol/L, pH 7.0) and then centrifuged at 12 500 g at 4℃ for 20 min. The resulting supernatant was collected as the enzyme source. The 3 mL reaction mixture contained 2.4 mL sodium phosphate buffer (66 mmol/L, pH 7.0), 0.2 mL enzyme source, and 0.3 mL of 50 mmol/L GSH. The reaction was initiated by the addition of 0.1 mL of 30 mmol/L CDNB. The OD value at 340 nm was recorded in a 25-s interval for 10 min. All reactions were done at 27℃.

2.6.5 *p*-Nitroanisole *O*-demethylase (ODM) assay: The *O*-demethylation of *p*-nitroanisole activity was assayed by using the methods of Qiu *et al*. (2003). A total of 50 larvae were homogenized in 5 mL of ice-cold phosphate buffer (0.1 mol/L, pH 7.8) and then centrifuged at 12 500 g at 4℃ for 20 min. The resulting supernatant was collected as the enzyme source. The reaction mixture contained 1 mL of enzyme preparation, 0.9 mL of sodium phosphate buffer (0.1 mol/L, pH 7.8), 1 mL of 3 mmol/L NADPH, and 0.1 mL of 50 mmol/L *p*-nitroanisole. The reaction was incubated at 25℃ for 30 min in a shaker and terminated with 0.5 mL of 1 mol/L hydrochloric acid. The product *p*-nitrophenol was then extracted with chloroform, and the chloroform fraction was back-extracted with 0.5 mol/L sodium hydroxide. The absorbance of the sodium hydroxide extract was recorded at 400 nm. And the quantity of the product was determined based on a *p*-nitrophenol standard curve.

2.7 Protein assay

Total protein content of the enzyme solution was determined by the Bradford method (Bradford, 1976) with minor revision. Enzyme solution (0.1 mL) was mixed with Coomassie brilliant blue solution (5 mL), and the mixture was incubated at 25℃ for 2 min. Then absorbance at 595 nm was read against blanks. The protein content was determined according to bovine serum albumin standard curve.

2.8 Statistical analysis

Bioassay data were analyzed by logit/probit dose response/mortality regression calculated using SPSS probit procedure (SPSS, version 16.0 for Windows, IBM, Chicago, IL). The data obtained from the experiments were statistically analyzed using one-way analysis of variance (ANOVA) followed by Fisher's LSD test at the 0.05 level. All percentage data were arcsine transformed before being subjected to analysis of variance.

3 RESULTS

3.1 Acute toxicity of cyantraniliprole and chlorantraniliprole to *P. xylostella* larvae

The LC_{50} values of cyantraniliprole and chlorantraniliprole at 48 h after treatment were 0.08 and 0.29 mg/L in the tested strain of *P. xylostella*, respectively (Table 1). And the LC_{50} value of chlorantraniliprole was about 3.63 times higher than that of cyantraniliprole. Based on the LC_{50} values, cyantraniliprole showed excellent insecticidal activity against *P. xylostella* when compared with chlorantraniliprole. The LC_{20} (0.03 mg/L) and LC_{50} (0.08 mg/L) were used to treat the insect in the following experiments. When the 3rd instar larvae were exposed to LC_{20} and LC_{50} of cyantraniliprole for 48 h, the corrected mortalities were 18.33% and 48.17%, respectively.

Table 1 Acute toxicity of cyantraniliprole against the 3rd instar larvae of *Plutella xylostella*

Insecticides	Regression equation	LC_{50} (95% FL) (mg/L)	Toxicity ratio	LC_{20} (mg/L)
Cyantraniliprole	$Y = 2.525 + 2.305X$	0.08 (0.05 – 0.11)	1.00	0.03 (0.02 – 0.05)
Chlorantraniliprole	$Y = 1.000 + 1.855X$	0.29 (0.19 – 0.40)	3.63	0.10 (0.05 – 0.16)

Toxicity ratio = LC_{50} of chlorantraniliprole/LC_{50} of cyantraniliprole.

3.2 Effects of cyantraniliprole exposure on biological characteristics of *P. xylostella*

Exposure of larvae to cyantraniliprole reduced the pupation rate, and adult emergence rate and extended the duration of the 4th instar (Table 2). When the 3rd instar larvae were treated with LC_{20} of cyantraniliprole, the pupation rate and adult emergence rate decreased by 9.73% and 10.31%, respectively, and the time for female and male larva needed to complete the 4th instar increased by about 0.84 and 0.56 d, respectively. When the 3rd instar larvae were treated with LC_{50} of cyantraniliprole, the pupation rate and adult emergence rate decreased by 27.50% and 21.39%, respectively, and the time for female and male larva needed to complete the 4th instar increased by about 1.66 and 1.59 d, respectively. In addition, cyantraniliprole had a considerable effect on *P. xylostella* pupae (Table 2). The pupal duration in both cyantraniliprole exposure treatments was obviously longer than the control and dose-dependent (female: $F = 29.15$; $df = 2,59$; $P < 0.05$; male: $F = 13.83$; $df = 2,45$; $P < 0.05$). Pupal weight was significantly lower in the treated groups as compared with the control (female: $F = 4.80$; $df = 2,68$; $P = 0.01$; male: $F = 11.46$; $df = 2,55$; $P < 0.05$), and there were no significant difference between the two insecticide treatments.

Table 2 Effects of cyantraniliprole on the duration of the 4th instar larva and pupa, pupal weight, pupation rate and adult emergence rate of *Plutella xylostella*

Cyantraniliprole concentration	Duration of the 4th instar (d)		Pupal duration (d)		Pupal weight (mg)		Pupation rate (%)	Adult emergence rate (%)
	Female	Male	Female	Male	Female	Male		
0 (Water)	2.95 ± 0.14 a	3.05 ± 0.19 a	4.89 ± 0.07 a	5.48 ± 0.10 a	6.16 ± 0.15 b	5.54 ± 0.11 b	94.80 ± 2.89 c	75.40 ± 2.40 c
LC_{20}	3.79 ± 0.15 b	3.61 ± 0.14 b	5.34 ± 0.08 b	5.81 ± 0.04 b	5.65 ± 0.12 a	5.15 ± 0.07 a	85.57 ± 1.96 b	67.63 ± 2.59 b
LC_{50}	4.61 ± 0.12 c	4.64 ± 0.24 c	5.72 ± 0.07 c	6.20 ± 0.14 c	5.63 ± 0.14 a	4.86 ± 0.12 a	68.73 ± 0.44 a	59.27 ± 1.55 a

Values are the means and standard deviations from three separate experiments. Means in the same column followed by different letters differ significantly ($P < 0.05$), based on the least significant difference (LSD) test. The same for Table 3.

Post-exposure effects of larval cyantraniliprole exposure were observed in adults (Table 3). Although there was no significant difference in the adult longevity between treated groups and the control (female: $F = 0.19$; $df = 2,26$; $P = 0.83$; male: $F = 0.19$; $df = 2,26$; $P = 0.83$), LC_{50} of cyantraniliprole significantly reduced the number of eggs laid per female (from around 103.00 to 57.60) and extended the pre-oviposition period (from around 0.98 to 1.56 d) as compared with the control and LC_{20} treatment.

3.3 Effects of cyantraniliprole exposure on enzyme activities in *P. xylostella*

After the 3rd instar larvae of *P. xylostella* were treated with LC_{20} and LC_{50} of cyantraniliprole , the CAT activity increased obviously in contrast with the control within 24 h, and this effect was dose-related (12 h: $F = 494.17$; $df = 2, 6$; $P < 0.05$; 24 h: $F = 360.62$; $df = 2,6$; $P < 0.05$). But no difference was found between treated groups and the control at 48 h after treatment ($F = 0.56$; $df = 2,6$; $P = 0.60$) (Fig. 1), so was the POD activity (Fig. 2).

Table 3　Effects of cyantraniliprole on adult longevity, pre-oviposition period, fecundity and egg hatch rate of *Plutella xylostella*

Cyantraniliprole concentration	Adult longevity (d)		Pre-oviposition period (d)	Number of eggs laid per female	Egg hatch rate (%)
	Female	Male			
0 (Water)	7.39 ± 0.44 a	8.64 ± 0.39 a	0.98 ± 0.04 a	103.00 ± 7.08 b	93.93 ± 0.55 a
LC_{20}	7.03 ± 0.39 a	8.96 ± 0.41 a	1.18 ± 0.09 a	109.60 ± 6.42 b	92.73 ± 0.52 a
LC_{50}	7.15 ± 0.47 a	8.93 ± 0.42 a	1.56 ± 0.15 b	57.60 ± 9.25 a	92.24 ± 0.82 a

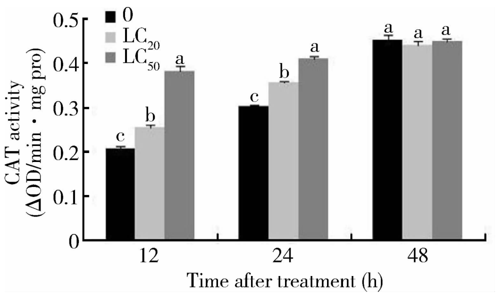

Fig. 1　Effects of cyantraniliprole on CAT activity in the 3rd instar larvae of *Plutella xylostella*

Data in the figure are means ± standard error of three separate replicates. Data of the same time after treatment marked with different letters differ significantly ($P < 0.05$) on the basis of the least significant difference (LSD) multiple comparison test. The same for the following figures.

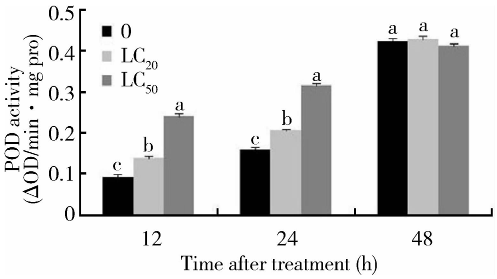

Fig. 2　Effects of cyantraniliprole on POD activity in the 3rd instar larvae of *Plutella xylostella*

The CarE activity in the 3rd instar larvae of *P. xylostella* had no difference between treated groups and the control at 12 h after treatment ($F = 0.98$; $df = 2, 6$; $P = 0.43$), and showed the positive correlation with the cyantraniliprole dosage (24 h: $F = 415.30$; $df = 2, 6$; $P < 0.05$; 48 h: $F = 92.05$; $df = 2, 6$; $P < 0.05$) (Fig. 3), but the activity in treated groups were higher than that in the control with time going by. The changes in GST and ODM activities had the similar phenomenon as that of the CarE acitivity (Figs. 4 and 5).

4 DISCUSSION

The anthranilic diamide insecticides are very popular because of its novel mode of action. In addition, they have been shown to be safe to non-target arthropods, including numerous beneficial insects and predatory mites (DuPont, 2008). But the insect pests have developed resistance due to the extensive use of these pesticides. Resistance in *P. xylostella* to chlorantraniliprole has been documented (Hu *et al.*, 2010; Wang and Wu, 2012), but now no report concerning about the resistance to cyantraniliprole. It is necessary to provide information that would enable us to use cyantraniliprole primarily to manage *P. xylostella* in crops. In this study, we found that low concentrations of cyantraniliprole (LC_{20} and LC_{50}) can affect not only the life traits of *P. xylostella*

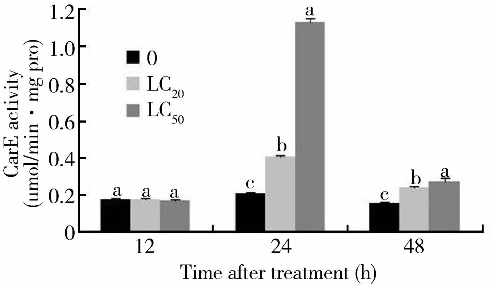

Fig. 3　Effects of cyantraniliprole on CarE activity in the 3rd instar larvae of *Plutella xylostella*

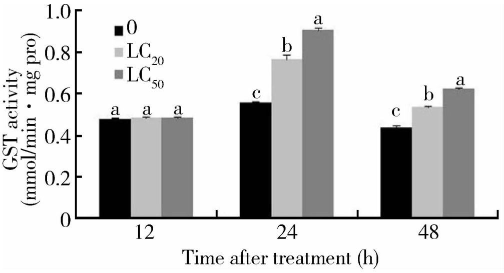

Fig. 4　Effects of cyantraniliprole on GST activity in the 3rd instar larvae of *Plutella xylostella*

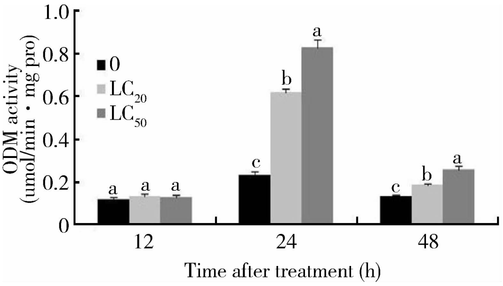

Fig. 5　Effects of cyantraniliprole on ODM activity in the 3rd instar larvae of *Plutella xylostella*

but also the enzyme activities.

4.1 Effects of cyantraniliprole on development of *P. xylostella*

Cyantraniliprole has been shown to be effective for whitefly control and *Tomato yellow leaf curl virus* suppression in field and greenhouse trials (Stansly *et al.*, 2010; Caballero *et al.*, 2013). But there is no related report on *P. xylostella*. In this study, we found a decrease in pupation rate and adult emergence rate, and an extension of the duration of the 4th instar after the 3rd instar larvae were treated with cyantraniliprole. Similarly, Han *et al.* (2012) found that chlorantraniliprole could reduce the pupation rate and adult emergence rate of *P. xylostella*. Lai and Su (2011) reported that chlorantraniliprole at LC_{30} (6.7 mg/L) and LC_{50} (12.7 mg/L) concentrations delayed the development of *S. exigua* larvae when they survived after initial exposure. There were many reports on reduction in pupal weight of insects after exposed to insecticides (Seth *et al.*, 2004; Pineda *et al.*, 2007; Liu *et al.*, 2008; Rodríguez Enríquez *et al.*, 2010). In cyantraniliprole exposed groups, we also found the pupal weight was reduced significantly compared with control. The reason may be that after exposure to cyantraniliprole, the larvae intake less food for optimal growth.

4.2 Effects of cyantraniliprole on the reproduction of *P. xylostella*

Insecticide hormoligosis is the term used to describe the phenomenon when low doses of insecticides increase fecundity and the reproduction parameters of insects, and cause outbreaks of insects (Mahmoudvand *et al.*, 2011). It was testified by Cohen (2006) that pest resurgence was induced by the stimulatory of a low dose range of an applied pesticide. Fujiwara *et al.* (2002) found that fenvalerate at low doses stimulated the reproductive potential of *P. xylostella*. Kerns and Stewart (2000) reported that treating *Myzus persicae* with low concentrations of bifenthrin increased its net reproductive rate and nymphal production. And resurgence of spider mite occurred after the application of synthetic pyrethroids (Gerson and Cohen, 1989). But in this study, hormoligosis was not observed in *P. xylostella* treated with cyantraniliprole. Our data showed that treatment of the 3rd instar larvae of *P. xylostella* with LC_{50} of cyantraniliprole could decrease adult fecundity by up to 44.7%. Similar results were obtained by Yin *et al.* (2008), who reported that spinosad reduced the number of eggs laid per female. Mahmoudv *et al.* (2011) argued that indoxacarb greatly reduced the reproductive capacity of *P. xylostella*. Pineda *et al.* (2009) also found the fecundity of *Spodoptera littoralis* was progressively decreased with increasing concentrations of azadirachtin. The differences between these and the present results suggested that cyantraniliprole may exert influences in a species-specific manner, revealing the selective insecticidal trait of the compound. Besides the different concentrations, the insect species may also lead to these different results. And intensive studies are needed to focus on the effects of low concentrations of cyantraniliprole on the continuous generations.

4.3 Effects of cyantraniliprole on enzyme activities in *P. xylostella*

CAT and POD are two important enzymes in insects and they mutually regulate to keep the free radical at a low level to avoid the damage to the body caused by them. And detoxification enzymes, such as CarE, GST and ODM, play important roles in the metabolism of toxic substances. The changes of enzyme activities could influence the effect of insecticides. In recent years, determining the activities of protective enzymes, detoxification enzymes and target enzymes, analyzing the sublethal effects of insecticide and then forecasting the population dynamics of insects have become the main subject of toxicology (Hyne and Maher, 2003; Desneux *et al.*, 2007). Therefore, to investigate the effects of cyantraniliprole on *P. xylostella* and prolong efficiency of cyantraniliprole, we measured the activities of CAT, POD, CarE, GST and ODM in the study. The activities of CAT and POD in the 3rd instar larvae fed on cabbage leaves exposed to cyantraniliprole were higher within 24 h, likely due to the self-protective reaction under the stresses caused by pesticides, but were the same as those in the control at 48 h after treatment. It might be explained that the superiority of protective enzymes in treated groups was transient. However, the changes in activities of detoxification enzymes showed an opposite trend, in which the activities in the treated groups were not significantly different from those in the control group within 12 h because of the less toxic substances in the body in relation to the little food intake, but then significantly higher than those in the control group because a great number of toxic substances were accumulated and much food was taken in. Wang *et al.* (2009) reported that ODM activity was significantly increased when the 5th instar larvae of *Spodoptera exigua* (Hübner) were fed with leaves treated with LD_9 of spinosad.

In summary, the present results suggest that cyantraniliprole has both lethal and sublethal effects on *P. xylostella*. The sublethal effects such as reduction in pupation rate, adult emergence rate and female fecundity may affect the population density of the next generation. Impacts such as delayed development of the 4th instar larvae and pupae may change the occurrence period of this pest. And the activities of detoxifying enzymes were related to insect resistance (Denholm and Rowland, 1992). So our results provide valuable information to develop rational use of cyantraniliprole in *P. xylostella* management. Further works are needed to evaluate the sublethal effects of cyantraniliprole on the 2nd or later generations.

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氰虫酰胺对小菜蛾生物学特性及相关酶活性的影响

刘 霞[1], 王红艳[2], 夏晓明[1], 乔 康[1], 王开运[1,*]

(1. 山东农业大学植物保护学院, 山东泰安 271018; 2. 山东棉花研究中心, 济南 250100)

摘要:【目的】小菜蛾 *Plutella xylostella*(L.)是全球十字花科植物上最重要的害虫。氰虫酰胺作为一种新型的邻甲酰胺基苯甲酰胺类杀虫剂有着独特的作用方式,而关于氰虫酰胺对小菜蛾的影响的报道几乎没有。【方法】本研究采用叶片药膜法研究室内条件下氰虫酰胺对小菜蛾生理生化的影响,饲喂小菜蛾含有氰虫酰胺药液(0, LC_{20}和LC_{50})的甘蓝叶片 48 h 后,观察小菜蛾的生物学特性及其相关酶活性的变化。【结果】氰虫酰胺对小菜蛾 3 龄幼虫的LC_{20}和LC_{50}分别为 0.03 和 0.08 mg/L。使用LC_{20}和LC_{50}浓度的氰虫酰胺处理小菜蛾 3 龄幼虫 48 h 后,对其影响表现为显著降低小菜蛾的蛹重、化蛹率和羽化率;明显延长 4 龄幼虫期和蛹期。采用这两个浓度氰虫酰胺处理小菜蛾 48 h,其保护酶(CAT 和 POD)活性在处理 24 h 内持续升高并且高于对照组;随后,活性继续升高但是与对照组没有差异(CAT: $P = 0.58$; POD: $P = 0.13$)。而其解毒酶(CarE, GST 和 ODM)活性在处理 12 h 内与对照组没有差异(CarE: $P = 0.43$; GST: $P = 0.54$; ODM: $P = 0.25$),但是随着取食时间的延长,其活性明显高于对照组。【结论】LC_{20}和LC_{50}浓度氰虫酰胺能够显著抑制小菜蛾的生长发育,对降低害虫虫口密度有一定的作用。同时这两个浓度氰虫酰胺还能够诱导小菜蛾体内解毒酶活性的升高,这将为田间合理施药,延缓害虫抗药性的产生提供理论依据。

关键词: 小菜蛾; 氰虫酰胺; 毒力; 酶活性; 生物学参数

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